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*STUDIES ON THE MECHANISM OF THE OXYGEN EFFECT ON THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES**

By Norman H. Giles, Jr., and Herbert Parkes Riley†

Biology Division, Oak Ridge National Laboratory

Communicated by Karl Sax, April 18, 1950

Previous experiments (Giles and Riley[1]) have demonstrated that the radiosensitivity of Tradescantia chromosomes, as measured by the occurrence of x-ray-induced aberrations in microspores, is markedly influenced by the amount of oxygen present. The frequencies of both interchanges and interstitial deletions observed four to five days following treatment are reduced if inflorescences are irradiated in gases such as nitrogen and helium and increased if exposures are made in pure oxygen instead of in air. On the basis of these experiments it was not possible to decide whether this effect of oxygen is exerted by way of the initial breakage mechanism, such that more breaks are produced by x-rays in the presence of oxygen, or whether the effect is on the recovery process, such that new reunions of broken ends are favored over restitutions. The present paper will discuss experiments performed to investigate certain aspects of this problem. Additional data will also be presented on the relation between aberration frequency and the percentage of oxygen present at the time of irradiation.

Experimental Methods.—Inflorescences of *Tradescantia paludosa* Anders. and Woodson, clone 5 (unless otherwise noted) of Sax were used. Aceto-carmine smear preparations of microspores at the first postmeiotic mitosis were made on the fourth and fifth days following irradiation and slides were scored for chromosomal aberrations—interchanges (dicentrics and centric rings) and interstitial deletions. In general, 50 or 100 cells from three to eight slides (each from a separate inflorescence) were scored and standard errors were calculated as previously. The same x-ray source was utilized—a Coolidge self-rectifying tube with tungsten target, operated at 250 kv and 15 ma. The inherent filtration was equivalent to 3 mm. of aluminum.

Irradiations were carried out as in earlier experiments in a lucite exposure chamber. However, in order to insure a more adequate control of the gas in the chamber and to facilitate a rapid removal or introduction of gas the experimental apparatus was redesigned. The new exposure chamber was placed directly in the x-ray machine and attached by pressure tubing and appropriate stopcock arrangements to a vacuum pump, a gas cylinder, and a mercury manometer. With this apparatus, all evacuations and introductions of gases could be performed directly with the inflorescences inside the exposure chamber. Evidence will be presented that the preexposure evacuations in a suction flask, as carried out in the earlier experiments, are unnecessary. Further, it is possible with this equipment to irradiate in a vacuum or under pressure and to introduce or remove gases during the period of irradiation. The presence of a manometer makes it possible to reproduce evacuation conditions and to detect possible unexpected changes of pressure in the system.

TABLE 1

COMPARATIVE EFFECTS OF VARIOUS PRETREATMENTS AND EXPOSURE-CHAMBER CONDITIONS ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOMAL REARRANGEMENTS IN TRADESCANTIA MICROSPORES

400 r AT 50 r/MIN.

PRETREATMENT	EXPOSURE CONDITIONS	NO. CELLS	INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I. D. PER CELL
Buds preevacuated and helium admitted	Helium in chamber	226	55	0.24 ± 0.03	51	0.23 ± 0.03
Buds preevacuated and helium admitted	Air in chamber	400	289	0.72 ± 0.04	323	0.81 ± 0.05
None	Helium in chamber	300	73	0.24 ± 0.03	72	0.24 ± 0.03

Results and Discussion.—A preliminary series of experiments was performed to determine whether the preexposure evacuations in a suction flask, as carried out in earlier experiments, were necessary to remove air enclosed around the anthers by the sepals and petals of the buds. For these tests two sets of buds were evacuated five times in a flask and helium permitted to diffuse in before irradiation. One set was placed in the exposure chamber (the original chamber was used), which was then evacuated and helium admitted; the other set was placed in the exposure chamber in air. The third set of buds was not preevacuated, but placed directly in the exposure chamber, which was then evacuated and helium admitted. All three sets received 400 r at 50 r/min. (table 1). It is clear that the pretreatment has no effect on the frequency of aberrations, but rather that the gas in which the exposure is made is the important factor. Evidently evacuation in the exposure chamber is sufficient to effect gas exchange in the buds. On the basis of these results, preevacuation of buds with a water pump was discontinued. Further, with the new chamber

and a vacuum pump it was possible to evacuate directly to considerably lower pressures than in the earlier experiments.

In the original experiments comparative exposures were made at only three oxygen levels—in nitrogen or helium (no oxygen), in air (ca. 21% oxygen), and in pure oxygen. It seemed of considerable interest to obtain further data in order to determine the quantitative relation between aberration frequency and percentage of oxygen during irradiation. Consequently, two separate experiments were carried out (in one clone 5 was used, in the other, clone 3 of Sax) in which exposures to a single x-ray dose—400 r at 50 r/min.—were made with seven different percentages of oxygen in the lucite chamber. The oxygen percentages were as follows:

TABLE 2

EFFECT OF VARIOUS PERCENTAGES OF OXYGEN ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES

ALL X-RAY EXPOSURES OF 400 r AT 50 r/MIN.

OXYGEN PERCENTAGE	CLONE USED	NO. CELLS	NO. INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I. D. PER CELL
0	Clone 3	450	120	0.27 ± 0.02	104	0.23 ± 0.02
	Clone 5	400	113	0.28 ± 0.03	88	0.22 ± 0.02
2	Clone 3	284	73	0.26 ± 0.03	95	0.33 ± 0.03
	Clone 5	315	82	0.26 ± 0.03	77	0.24 ± 0.03
10	Clone 3	425	304	0.72 ± 0.04	305	0.72 ± 0.04
	Clone 5	200	152	0.76 ± 0.06	162	0.81 ± 0.06
21	Clone 3	350	322	0.92 ± 0.05	392	1.12 ± 0.06
(Air)	Clone 5	150	118	0.79 ± 0.07	116	0.77 ± 0.07
60	Clone 3	303	283	0.93 ± 0.06	364	1.20 ± 0.06
	Clone 5	200	181	0.91 ± 0.07	196	0.98 ± 0.07
100	Clone 3	250	249	1.00 ± 0.06	318	1.27 ± 0.07
	Clone 5	150	148	0.99 ± 0.08	159	1.06 ± 0.08
100 (at an absolute pressure of 1500 mm. Hg)	Clone 3	225	245	1.09 ± 0.07	279	1.24 ± 0.07

0% (irradiation in pure—99.8%—helium); 2% (+98% helium); 10% (+90% helium); 21% (air); 60% (+40% helium); 99.5% (pure oxygen from a commercial cylinder); and pure oxygen at an absolute pressure of 1500 mm. of mercury (approximately 760 mm. above normal atmospheric pressure at Oak Ridge). Before each exposure, inflorescences were placed in the chamber, which was then evacuated to approximately 1 to 2 mm. of mercury, and the particular gas or gas mixture admitted. This procedure was repeated five times. Following irradiation, the inflorescences remained in the chamber for approximately ten minutes in the same gas and were then removed to air. The results of these experiments are presented in table 2 and figure 1. There is good agreement between the two

experiments except for the two points in air, where, for some unexplained reason, the values obtained for clone 3 are considerably higher than expected. The data indicate that there is a very rapid rise in aberration frequency between 2 and 20% oxygen, after which a gradual increase apparently occurs. The significance of the fact that essentially the same aberration frequencies were obtained in exposures made in pure (99.8%) helium and 2% oxygen (+98% helium) is not yet clear. This may mean that not all of the dissolved air is removed from the tissues by the evacuation procedure. It is also desirable to check further the accuracy of the reported percentage of oxygen in the gas mixture used (obtained from a

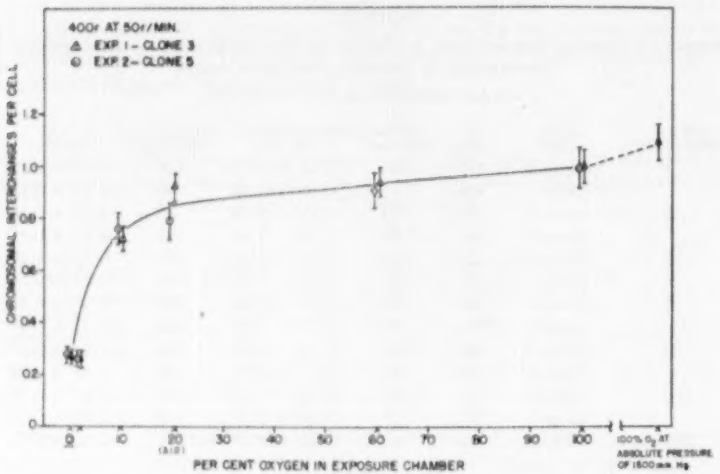

FIGURE 1

Relation between percentage of oxygen in exposure chamber and frequency of chromosomal interchanges per cell in Tradescantia microspores. All exposure to one x-ray dosage—400 r at 50 r/min. Two separate experiments, one with clone 3 and one with clone 5, as indicated.

commercial source). The general problem of the effect of oxygen during irradiation at low oxygen tensions is being investigated further.

The major problem requiring further investigation was concerned with the mechanism of the oxygen effect in increasing aberration frequencies—whether this effect resulted from a higher initial production of chromosome breaks, or from a relative increase in new reunions as opposed to restitutions of broken ends during the recovery process. It is clear from the earlier studies of Sax,[2] Marinelli, Nebel, Giles and Charles,[3] and Lea and Catche-

side,[4] that in Tradescantia there is an appreciable time interval between the production of a break and its disappearance, either by restitution or new reunion (interchange). The average time of restitution for the majority of the breaks has been estimated by Lea[5] to be about four minutes. Thus it would appear to be experimentally feasible to determine whether the oxygen effect is on initial breakage or on recovery if these two processes can be made to take place under different conditions with respect to the presence or absence of oxygen. The following series of comparative exposures (all at a single constant dosage of 300 r at 300 r/min.) was accordingly carried out. The exposure conditions about to be described are summarized in table 3. In series 1 and 2 buds were evacuated for five minutes at 1 to 2 mm. of mercury and irradiated in vacuum. Series 1 was maintained in vacuum in the exposure chamber for ten minutes following irradiation. In series 2 pure oxygen was introduced into the exposure chamber to an absolute pressure of 1500 mm. of mercury immediately following irradiation. The introduction of oxygen to this pressure was effected within three seconds following cessation of the irradiation and the buds were maintained in oxygen for ten minutes. In series 3 and 4

TABLE 3

EXPERIMENTS ON THE MECHANISM OF THE EFFECT OF OXYGEN IN INCREASING THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES. FOR FURTHER DISCUSSION, SEE TEXT.

ALL EXPOSURES: 300 r AT 300 r/MIN.

SERIES NO.	PRE-TREATMENT CONDITIONS	EXPOSURE CONDITIONS	POSTTREATMENT CONDITIONS	NO. CELLS	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS PER CELL
1	Buds in vacuum	Vacuum	Vacuum—10 min.	880	0.12 ± 0.01	0.11 ± 0.01
2	Buds in vacuum	Vacuum	Oxygen introduced (within 3 secs.) to 1500 mm. Hg—10 min.	700	0.09 ± 0.01	0.10 ± 0.01
3	Buds in oxygen	Oxygen at 1500 mm. Hg	Oxygen at 1500 mm. Hg—10 min.	150	0.70 ± 0.07	0.83 ± 0.07
4	Buds in oxygen	Oxygen at 1500 mm. Hg	Evacuation (within 25 sec.); vacuum 10 min.	200	0.72 ± 0.06	0.85 ± 0.07
5	Buds in vacuum	1st 30 sec., vacuum. 2nd 30 sec., oxygen introduced (within 3 sec.) to 1500 mm. Hg	Evacuation (within 25 sec.); vacuum —10 min.	350	0.39 ± 0.03	0.50 ± 0.04
6	Buds in oxygen	1st 30 sec., oxygen at 1500 mm. Hg. 2nd 30 sec., evacuated (within 25 sec.) to 1 to 2 mm. Hg	Oxygen introduced (within 3 sec.) to 1500 mm. Hg—10 min.	518	0.61 ± 0.03	0.59 ± 0.03

buds were placed in oxygen (by the usual procedure for evacuation and introduction of gas) at an absolute pressure of 1500 mm. of mercury and irradiated in oxygen. Series 3 was maintained in oxygen for ten minutes after irradiation. In series 4 the chamber was evacuated immediately following irradiation. This evacuation to 1 to 2 mm. of mercury was accomplished within 25 seconds following the cessation of the irradiation and the vacuum was maintained for ten minutes. In series 5 buds were evacuated as in series 1 and 2 and irradiation was commenced with the buds in a vacuum. At the end of 30 seconds of exposure, oxygen was introduced into the chamber, without interrupting the irradiation, to an absolute pressure of 1500 mm. of mercury (within three seconds) and after the cessation of the total irradiation time of 60 seconds, the chamber was immediately evacuated to 1 to 2 mm. of mercury (within 25 seconds) and the buds kept in vacuum for ten minutes. In series 6 buds were placed in oxygen as in series 3 and 4 and irradiation was commenced with the buds in oxygen. At the end of 30 seconds of exposure the chamber was evacuated, without interrupting the irradiation, to 1 to 2 mm. of mercury (within 25 seconds) and after the cessation of the total irradiation time of 60 seconds oxygen was reintroduced to an absolute pressure of 1500 mm. of mercury (within three seconds). The exposure of 300 r/min. for one minute was selected to make the total time of exposure as short as feasible compared to the average time for restitution, since restitution takes place during the period of irradiation also. The data obtained from these exposures are presented in table 3.

It is clear from the first comparison (series 1 and 2) that the addition of oxygen immediately after irradiation does not increase the frequency of aberrations. Such an increase would be expected if there were an effect of oxygen on the reunion process. Nor does the removal of oxygen (series 3 and 4) after irradiation result in a lower aberration frequency. In both comparisons the observed aberration frequency apparently depends on the presence or absence of oxygen at the time of irradiation. The additional experiments were included to test this point further and also to exclude the possibility that the addition or removal of oxygen (to or from the cells themselves) after irradiation was not accomplished rapidly enough to detect an effect on the reunion process if such an effect existed. In series 5, it is evident that the addition of oxygen during irradiation results in a marked increase in aberration frequency. There is apparently an almost immediate entrance of a considerable amount of oxygen into the cells of the anthers at the beginning of the final 30 seconds of irradiation (the period of three seconds indicated is the time required to introduce oxygen to a pressure of 1500 mm. of mercury; considerably less time is probably required for the introduction of sufficient oxygen to produce an almost maximal increase in aberration frequency). In series 6 there is an appreciable decrease in aberration frequency accompanying the removal of

oxygen during the last 30 seconds of radiation. The fact that this decrease is not as marked as the increase noted on the addition of oxygen in series 5 appears to be quite reasonable, since a considerably longer period is required to evacuate the chamber than to introduce oxygen.

On the basis of the data which have been presented it is clear that the postirradiation presence or absence of oxygen has no effect on the recovery process. It seems reasonable to conclude also that there is no effect of oxygen on the recovery process occurring during irradiation, which in these experiments occupies a relatively small fraction of the total recovery period. Pretreatment of buds in the presence or absence of oxygen has no effect; oxygen must be present during the actual X-ray exposure to produce an increase in aberration frequency. Furthermore, the experiments in which oxygen is introduced during irradiation indicate that its effect is immediate. It thus appears that the effect of oxygen must be exerted on the breakage mechanism. However, it seems probable that this effect is actually an indirect one. The most likely hypothesis seems to be that when dissolved oxygen is present during irradiation in the largely aqueous medium in cells, some substance resulting from the radiodecomposition of water containing oxygen is formed which causes an increased aberration frequency. On the basis of their experiments with *Vicia faba*, Thoday and Reed[6] suggest that this substance may be hydrogen peroxide. This possibility is supported by the results obtained in the present experiments relating aberration frequency to percentage of oxygen at the time of irradiation. This relation (fig. 1) is generally similar to that for the yield of hydrogen peroxide when water containing increasing concentrations of oxygen is subjected to X rays (Bonet-Maury and Lefort[7]). If it is assumed that hydrogen peroxide is in fact the substance indirectly responsible for the increased aberration frequency obtained in oxygen, it appears likely that such an increase would result from a higher frequency of initial breakage, rather than from an effect on the recovery process. This conclusion is supported by the observations of Baker and Sgourakis[8] that oxygen increases the yield of X-ray induced sex-linked lethal mutations in *Drosophila*, where there is no evidence that a recovery process is involved. However, the possibility cannot yet be excluded that in Tradescantia an intermediate radiation product such as hydrogen peroxide might produce an effect by modifying the behavior of broken ends, themselves produced by direct radiation action, and thus influence the restitution process.

Summary.—Further experiments have been performed on the effect of oxygen in increasing the radiosensitivity of Tradescantia microspore chromosomes. Exposures of inflorescences to a single constant x-ray dose, but in atmospheres containing seven different percentages of oxygen indicate that there is a rapid rise in aberration frequency between 2 and 21% oxygen, with a gradual increase thereafter. Further studies are being made to clarify the effect of oxygen at levels between zero and two

per cent. Other experiments have been performed to determine whether the oxygen effect is exerted by way of the initial breakage mechanism or on the reunion process. These consisted of comparative exposures to a single dose of 300 r in one minute of inflorescences in a vacuum or in oxygen with the addition or removal of oxygen either immediately after or during part of the irradiation period. In addition, buds were pretreated in the presence and absence of oxygen before exposure to X rays. These experiments show that the presence of oxygen during the actual exposure to X rays rather than during the pre- or postirradiation period is the important factor, thus indicating that oxygen alone does not influence the recovery process. It seems likely that the oxygen effect is an indirect one, resulting from the production during irradiation in oxygen of some substance such as hydrogen peroxide. Although it appears probable that the effect of such a substance on aberration frequency would result from an increased production of chromosome breaks, the alternative possibility, that such a substance might modify the restitution process, cannot yet be excluded.

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[†] Present address: Department of Botany, University of Kentucky, Lexington, Ky.

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THE ORIGIN AND BEHAVIOR OF MUTABLE LOCI IN MAIZE

BY BARBARA MCCLINTOCK

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION, COLD SPRING HARBOR, NEW YORK

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In the course of an experiment designed to reveal the genic composition of the short arm of chromosome 9, a phenomenon of rare occurrence (or recognition) in maize began to appear with remarkably high frequencies in the cultures. The terms mutable genes, unstable genes, variegation,

mosaicism, mutable loci or "position-effect" have been applied to this phenomenon. Its occurrence in a wide variety of organisms has been recognized. The most extensive investigations of this phenomenon have been undertaken in *Drosophila melanogaster*.[1] In this organism, the conditions associated with the origin of genic instability have been well defined. The part played by the heterochromatic materials of the chromosomes, in inducing and controlling the type of variegation and its time and frequency of occurrence, has been established. It has not been generally recognized that the instability of genic expression in other organisms may be essentially the same as that occurring in Drosophila.

As stated above, a large number of mutable loci have recently arisen in the maize cultures and are continuing to arise anew. The loci affect variegation for many different kinds of plant characters, each locus being concerned with a particular character or occasionally several characters. Some of these loci are *c*, *yg*$_2$, *wx*, *a*$_2$, *y*, *pyd*, which are well-investigated units in maize.[2] Others involve previously unknown genetic units. The same types of genic instability appearing in the maize cultures have been described in many other organisms. The behavior of these new mutable loci in maize cannot be considered peculiar to this organism. The author believes that the mechanism underlying the phenomenon of variegation is basically the same in all organisms. The reasons for this conclusion will be made apparent in the discussion.

The initial appearance of the burst of newly arising mutable loci occurred in the progeny coming from the self-pollination of about 450 plants which had each undergone a series of events in their early development where the short arm of chromosome 9 was subjected to drastic structural modifications. These events took place during the "chromosome type" of breakage-fusion-bridge cycle.[3] The modifications that this mechanism produces are: one or more duplications of segments of the short arm, deficiencies of one or more segments of various lengths, structural modifications of the heterochromatic knob substance, duplications of the knobs with or without structural modifications, and various combinations of these several types of modifications. The chromosome complement of over 150 of these plants were examined at pachytene to determine the nature of the structural modifications that had occurred. In addition to the modifications of the short arm of chromosome 9 listed above, some of the plants had other modifications, many of which are particularly significant because they involve the substances in the chromosome that are believed to be responsible for the origin and behavior of mutable loci—the heterochromatic knobs and centromeres. Altogether, 48 such structural modifications have been analyzed, most particularly in the above-mentioned plants but also in some other plants that had received a chromosome 9 with a newly broken end. Fourteen involved modifications of chromo-

some 9 other than those listed above (telocentric chromosomes, isochromosomes, extra chromosomes 9 with particular modifications, etc.). Four arose from fusion of the centromere of chromosome 9 with the centromere of another chromosome. Four resulted from fusion of the knob substance of the short arm of chromosome 9 with the centromere of chromosome 9. Twenty-four resulted from fusions of the knob substance of the short arm of chromosome 9 with other regions in the chromosome complement: eighteen were with other knobs or with regions very close to these knobs, four were insufficiently analyzed as to the positions of the fusion, and two did not involve a known knob region. In two cases, inversions were present in other chromosomes. The regions involved were the knob and centromere in one of these chromosomes and the nucleolus organizer and the centromere in the other chromosome. There can be no question that these "spontaneous translocations" are nonrandom with respect to the location of the breaks and fusions. The heterochromatic knob and centromere regions are mainly involved.

In the cultures arising from self-pollination of the plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early development, about 40 different mutable loci were recognized. The majority of such mutable loci could not have been present in the parents of these plants, for the stocks from which they arose had been under investigation for some years without showing evidence of the presence of such a large number of unstable loci. It was concluded, therefore, that either some part of the mechanism concerned with the breakage-fusion-bridge cycle or some of the structural modifications resulting from it were responsible for conditions that produced this burst. That some of the mutable loci were located in or associated with chromosome 9 was realized in the first tests. Other mutable loci, on the other hand, did not show any obvious association with chromosome 9.

The mutable loci fall into two major classes: (1) those that require a separate activator factor for instability to be expressed, and (2) those that are autonomous with respect to the factor that controls the onset of mutability. They also may be subdivided on a quite different basis. This is related to the types of expression of the mutations that occur. The following types are present: (*a*) Changes from the mutant to, or close to, the wild-type expression. After such a mutation, the locus may be permanently stabilized. It may no longer show evidence of the instability phenomenon. (*b*) A second group, similar to (*a*) except that the mutation to wild-type does not produce stability of the locus. The wild-type-producing locus, in turn, may mutate to give the recessive expression. (*c*) A third type where the mutations give rise to a series of alleles of the affected loci. These alleles are distinguished by different degrees of quantitative expression of the normal phenotype. Most of these are

relatively stable; only rarely does instability again appear. (*d*) A fourth type, similar to (*c*). Most of the alleles, however, are not stable for they, in turn, can mutate in the direction of a higher or lower grade of quantitative expression of the phenotype. Mutable loci showing these different types of expression of mutation are found in both the major classes, that is, in the activator-requiring class and in the autonomous class.

The accumulated observations and data from a study of a number of these mutable loci are so extensive that no short account would give sufficient information to prepare the reader for an independent judgment of the nature of the phenomenon. It is realized that this is unfortunate. Manuscripts giving full accounts of some of this phenomenon are in preparation. Since this task will require much time to fulfill, the author has decided to present this short account of the general nature of the study, and the conclusions and interpretations that have been drawn. In this account only short summaries will be given of some of the pertinent information that has led to the conclusions to be presented. These conclusions are concerned with the origin of mutable loci, the events occurring at these loci that result in a change in phenotypic expression, the reasons for changes in the frequency of visible mutations at these loci, the factors controlling the time when mutations will occur, the production of mutations at the a_1 locus in maize without *Dt* being present, and heterochromatin as the probable controlling factor.

A fortunate discovery was made early in the study of the mutable loci which proved to be of singular importance in showing the kinds of events that are associated with their origin and behavior. A locus was found in the short arm of chromosome 9 at which breaks were occurring in somatic cells. The time and frequency of the breakage events occurring at this *Ds* (Dissociation) locus appeared to be the same as the time and frequency of the mutation-producing events occurring at some of the mutable loci.[4] An extensive study of the *Ds* locus has indicated the reason for this relationship and has produced the information required to interpret the events occurring at mutable loci. It has been concluded that the changed phenotypic expressions of such loci are related to changes in a chromatin element other than that composing the genes themselves, and that mutable loci arise when such chromatin is inserted adjacent to the genes that are showing the variegated expression. The events occurring to this inserted chromatin are reflected in a changed expression of the neighboring genes, or sometimes in a loss of these genes. It is the inserted material that is undergoing the "mutational" events. The *Ds* locus is composed of this kind of material.

Various types of alterations are observed as the consequence of events occurring at the *Ds* locus. Some of these alterations resemble the effects produced by x-rays, ultra-violet light, chemicals, etc. They involve

chromosome breakage and fusion. The breaks are related, however, to events occurring at this one specific locus in the chromosome—the *Ds* locus. The *Ds* designation was given to this locus because the dissociation, now known to be related to dicentric and associated acentric chromatid formation, was recognized before the other events occurring at *Ds* had been disclosed. Some of the events occurring at *Ds*, when considered without reference to all the known events, would not by themselves suggest that changed conditions at this locus are associated with a breakage-inducing phenomenon. All of them can be explained, however, by the assumption that one kind of alteration of the inserted chromatin (the chromatin of the *Ds* locus) takes place, and that the various kinds of changes observed represent consequences of this one altered condition. This condition is assumed to be a stickiness of the materials composing the *Ds* locus, which arises only at precise times in the development of a tissue. The control of the timing of this changed condition will be considered shortly. The reasons for assuming the change to be a stickiness will be obvious from the following list of known events that involve the *Ds* locus. These are: (1) Dicentric chromatid formation with fusion of sister chromatids at the location of *Ds*. This is accompanied by formation of an acentric fragment composed of the two sister segments of this arm, from *Ds* to the end of the arm. (2) Loss of detectable *Ds* activity without visible alteration of the chromosome. In some cases, the loss of *Ds* activity is presumably due to loss of the locus itself. (3) Deletions of chromatin segments of various lengths adjacent to *Ds*, usually with concomitant loss of *Ds* activity but occasionally without loss of this activity. (4) Reciprocal translocation involving chromosome 9 in which one breakage point is at *Ds*. (5) Duplications of segments of chromosome 9, inversion or ring chromosome formations involving chromosome 9 with one break at the *Ds* locus. (6) Transposition of *Ds* activity from one position to another in the chromosomal complement with or without an associated gross chromosomal rearrangement. (7) Changes at the *Ds* locus itself which result in precise changes in the relative frequency of occurrence of the above types of events in future cell and plant generations. This last event, which is of considerable importance, has been termed "change in state" of the *Ds* locus. From a study of the progression of changes in state of *Ds* through cell and plant generations, it appears that the various states may reflect the quantity of the inserted chromatin, the *Ds* loci with larger quantities of this material showing a high frequency of consequences (1), (3), (4), (5) and (6) above, and those with less of this material showing high frequencies of consequence (2) above.

It is from the transpositions of *Ds* that some of the new mutable loci may arise. The mechanism of transposition has received considerable study. Some cases of transposition of *Ds* are associated with a gross

chromosomal rearrangement. In these cases, two chromosome breaks occur to give rise to the rearrangement; one break marks the known position of *Ds* in the chromosome, before the rearrangement occurred, and the second break marks the new position of *Ds* activity. Sister chromatids are affected at each of these two positions of breakage. It has been determined for several of these cases that the appearance of *Ds* activity at the new position most probably arose at the time of origin of the gross chromosomal rearrangement. One case of transposition of *Ds* has been of particular importance because it illustrates how new mutable loci, associated with changes in genic expression, can arise. This transposed *Ds* locus appeared in a single gamete of a plant carrying chromosomes 9 with the dominant *C* allele. This gamete carried a *Ds* locus that had been transposed from a known position in the chromosome 9 to a new position in the same chromosome. The chromosome having *Ds* at this new position was morphologically normal in appearance. This new position of *Ds* corresponded to the known location of *C* (*C*, colored aleurone, dominant to *c*, colorless aleurone). All of the above-enumerated events were now occurring at this new position. Significantly, the appearance of *Ds* activity at this new location was correlated with the disappearance of the normal action of the *C* locus. The resulting phenotype was the same as that produced by the known recessive, *c*. It has been determined from previous studies that a deficiency of the *C* locus will give rise to a *c* phenotype. That the *c* phenotype in this case was associated with the appearance of *Ds* at the *C* locus, and was not due to a deficiency, was made evident because mutations at this locus from a *c* to a full *C* phenotypic expression occurred. It could be shown that when *C* action reappeared, the *Ds* action concomitantly disappeared from this locus. The restored action of *C* was permanent; no further *Ds*-type events occurred at this *C* locus. In most cases, the event giving a restored *C* action did not result in an altered morphology of chromosome 9. Loss of *Ds* activity without concomitant structural alterations of the chromosome result from event (2) above.

The other enumerated events associated with *Ds* activity were also occurring at this mutable *c* locus. The dicentric chromatid formations were not associated with the appearance of a *C* phenotype, suggesting that the inserted inhibiting material composing *Ds* may be situated proximal to the *C* locus. Several cases of transposition of *Ds* from this location to still another location in the short arm of chromosome 9 were recognized. In each case, a restored *C* action was associated with a disappearance of *Ds* activity at the *C* locus and the appearance of *Ds* activity at the new position. The changes in state of *Ds* at this mutable *c* locus (event (7) above) are particularly significant since it has been determined that a specific change in state of *Ds* is often accompanied by a specific change in the frequency of *c* to *C* mutations.

The origin and behavior of this mutable *c* locus has been interpreted as follows: Insertion of the chromatin composing *Ds* adjacent to the *C* locus is responsible for complete inhibition of the action of *C*. Removal of this foreign chromatin can occur. In many cases, the mechanism associated with this removal results in restoration of the former genic organization and action. The *Ds* material and its behavior are responsible for the origin and the expression of instability of the mutable *c* locus. The mutation-producing mechanisms involve only *Ds*. No gene mutations occur at the *C* locus; the restoration of its action is due to the removal of the inhibiting *Ds* chromatin. The possible nature of the inserted material will be considered later.

In the cultures having *Ds*, other mutable loci continue to arise. They show types of behavior similar to that described for the mutable *c* locus. This mutable *c* locus (called *c-m1* because it was the first of the mutable *c* loci isolated in these cultures) belongs to the (*a*) group of mutable loci. In some of the progeny of the original self-pollinated cultures, other mutable *c* loci have arisen from previously normal *C* loci. One of these, *c-m2*, shows the type (*c*) expression of variegation, which differs markedly from that shown by *c-m1*. A wide range of quantitative expression, for at least two different reactions associated with aleurone pigment formation, appears as the consequence of various mutations at this locus. The intermediate alleles, full wild-type alleles and some alleles showing even stronger phenotypic expressions than the wild-type from which it arose, are produced by mutations at *c-m2*. The mutations are often expressed as twin sectors, the depth of color in one sector being greater than that in the sister sector. These twin sectors may reflect a single mutation-producing event at the *c-m2* locus that involved both sister chromatids. It has also been determined that chromosome breakage may occur at this locus.

The phenotypic expressions resulting from mutations of *c-m2* and *c-m1* are clearly quite different. That this difference may be related to differences in the inserted chromatin is suggested by the appearance of a mutable *wx* locus arising from a *Wx* locus in a gamete of a plant carrying *c-m2* (*Wx*, starch of endosperm stains blue with iodine; *wx*, recessive allele, starch stains red with iodine; located in short arm of chromosome 9, proximal to *C*). The type of variegation expressed by this mutable *wx* locus (*wx-m1*) is strikingly similar in all respects to that occurring at *c-m2*. It could not be determined in this case that transposition to the *Wx* locus of the same inhibiting substance that induced *c-m2* had occurred. Such an event is suspected from the known transposition capacities of this material.

In this report, *Ds*, *c-m1*, *c-m2* and *wx-m1* have been used as illustrations of newly arising mutable loci because all of them require an activator and

all respond to the same activator. This activator has been designated *Ac*. Extensive studies of *Ac* have shown that it is inherited as a single unit. It shows, however, a very important characteristic not exhibited in studies of the inheritance of the usual genetic factors. This characteristic is the same as that shown by *Ds*. Transposition of *Ac* takes place from one position in the chromosomal complement to another—very often from one chromosome to another. Again, as in *Ds*, changes in state may occur at the *Ac* locus These changes in state are of two main types: either changes that resemble the known effects produced by different doses of the *Ac* locus from which it was derived, or changes that result in a decidedly altered time of action and dosage response of *Ac*. *Ac* may be detected and its action studied by observing the mutations occurring at the mutable loci requiring its presence for mutability to be expressed. It should be emphasized that when no *Ac* is present in a nucleus, no mutation-producing events occur at *c-m1*, *c-m2* or *wx-m1;* nor are any chromosome breakage events detected at *Ds*, for no such events occur. As an example of this interaction it may be stated that *c-m1* has been maintained in cultures having no *Ac* locus for several generations, and has given completely colorless aleurone with no evidence of *c* to *C* mutations. Similarly, the various quantitative alleles arising from mutations of *c-m2* or *wx-m1* may be maintained without giving mutations, if *Ac* is removed from the nucleus by appropriate crosses. Thus a series of stable recessive mutations or stable alleles of a mutable locus may be isolated and maintained (if the chromosome complement is normal, see below). When *Ac* is returned to the nucleus, however, instability may again appear.

The dosage action of *Ac* may be studied in the diploid plant or in the triploid endosperm tissue of the kernels. When marked dosage effects are produced by a particular state of *Ac*, they are registered alike in both the plant and the endosperm tissues; the higher the dose of *Ac*, the more delayed is the time of occurrence of mutations at the *Ac*-controlled mutable loci. *Ac* determines, therefore, not only the mutation process at these mutable loci but also the time at which the mutations occur, the different states of *Ac* giving different times of occurrence in 1, 2 or 3 doses. The action of *Ac* on the mutable loci it controls has been described. It is believed that this action produces a stickiness of the inhibiting materials adjacent to the affected loci. With reference to *Ds*, the observed consequences of this stickiness have been enumerated. This physical change probably takes place in the inserted inhibiting materials at all the *Ac*-controlled mutable loci at the same time in the same cell. This latter conclusion rests on the observation that mutations occur concomitantly at two or more *Ac*-controlled mutable loci when these are present in the same nucleus. The similarity in the type of inheritance and the behavior of *Ds* and *Ac* has been indicated above. Another similarity is that changes

in state, loss or transposition of *Ac* occur at the same time that changes take place at the *Ac*-controlled mutable loci. It would appear that the changes in the physical properties of the specific inhibiting chromatin at the mutable loci and at *Ac* itself are of the same nature, and that all are expressions of the primary genetic action of the material composing *Ac*. It is suspected that *Ds* and *Ac* are composed of the same or similar types of material. The possible composition of this material will be considered shortly.

The study of *Ac* and the *Ac*-controlled mutable loci has made it possible to interpret the many patterns of variegation exhibited by mutable loci. The variegated pattern is an expression of the time and frequency of occurrence of visible changes in the phenotype. The frequency of appearance of a visible mutation need not reflect the frequency of the events that occur at a mutable locus, as the study of *c-m1* has clearly revealed. The visible mutations reflect only the frequency of one or several particular consequences of one primary type of event occurring to the inhibiting material adjacent to the affected gene. The changes in state of this inhibiting material that arise as one of the consequences of the primary event, lead to changes in the relative frequency of the consequences of this event when it again occurs in future cell and plant generations. Such changes in state are reflected either in increases or decreases in the relative frequency of appearance of visible mutations. The study of *Ac* has indicated the nature of the control of the time when the mutations will occur at these mutable loci. The different doses of *Ac* together with the changed states of *Ac* control the time of occurrence of these mutations. The changes in time of occurrence of visible mutations are thus reflections of changes in dosage or changes in state of *Ac*.

The mutable loci that require no activator show the same kinds of expression of variegation as do the activator-requiring mutable loci. It has been shown that the changes occurring at *Ac* are much the same as those occurring at *Ds*. Thus, *Ac* or *Ac*-like loci, could be responsible for the origin of new mutable loci when transposed to a position adjacent to a gene whose inhibited action could be detected by a visible change in phenotype. Dosage action could be exhibited by such autonomous mutable loci, as well as various "changes in state," reflected by changes in the phenotypic expression and the time and frequency of occurrence of visible mutations of the affected genes. The study of the behavior of *Ds* in its several states makes it possible to reinterpret the variegation patterns in Drosophila, which in some cases appear to be associated with loss of segments of chromosomes and in other cases appear to be associated with changes in the degree of action of the genes involved. It also makes it possible to interpret the reported "position-effect" in Oenothera, because the events responsible for the changes in phenotype and the appearance of

duplications and deficiencies in this organism appear to be the same or similar to those described for *Ds* in maize.[5]

The possible composition of *Ac* may now be considered. Until recently, the investigation was not focused on this problem. It is believed, however, that this material is probably heterochromatin. This statement is based, in part, on the evident homologies in the expression of variegation in maize and Drosophila, but is more convincingly suggested by the results of a preliminary experiment focused on the induction of mutations at the a_1 locus in maize when the known *Dt* (Dotted) locus is absent. The action of *Dt* in chromosome 9 on the a_1 locus in chromosome 3 is very much the same as the action of *Ac* on the mutable loci it controls.[6] The similarities are too great to be dismissed as being due to causally unrelated phenomena. The *Dt* locus activates the a_1 locus; mutations to higher A_1 alleles occur (A_1, colored aleurone; a_1, colorless aleurone, recessive to A_1). Without *Dt* in the nucleus, a_1 has been shown to be completely stable. *Dt* is located in the *heterochromatic* knob terminating the short arm of chromosome 9. The suspicion is immediately aroused: Is *Dt* action caused by some modification of the heterochromatic knob in chromosome 9? If so, could this modification be produced anew by subjecting a chromosome 9 to the breakage-fusion-bridge cycle? Would the effective alterations of the knob arise directly because of the induced changes, or would they be produced secondarily by some other induced structural alteration, either within the short arm of chromosome 9 or elsewhere, that would upset, in some way, the normal functioning of the knob substance and thus bring about an alteration in its action? This last question is pertinent because some of the structural alterations in Drosophila appear to affect the functioning of the centrically placed heterochromatin. For example, some of the Minutes bring about chromosome elimination and "somatic-crossingover," both of which may well be related to adhesions of specific heterochromatin that occur at certain times in development.[7] To answer the above questions, plants homozygous for a_1 and having no *Dt* locus (designated *dt* by Rhoades) were crossed by plants similarly constituted with reference to a_1 and *dt* but carrying a rearrangement of the short arm of chromosome 9 that would introduce a chromosome 9 with a newly broken end into many of the primary endosperm nuclei in the given cross.[8] Breakage-fusion-bridge cycles involving such a chromosome 9 with a newly broken end would occur during the development of the kernels. Some of these broken chromosomes 9 would carry a knob, and this knob could then be subjected to modifications as a consequence of the breakage events. If some of these modifications gave rise to the same conditions that were present at *Dt*, mutations from a_1 to A_1 could appear in some of the kernels resulting from the cross. A large number of crosses of this type were made. The results were positive with respect to inducing mutations of a_1 to A_1. A small

number of the kernels resulting from these crosses showed mutations of a_1 to A_1. Often, only a single small A_1 spot was present on the kernel. Several of the kernels, however, had a pattern of mutations of a_1 to A_1 that was indistinguishable from that produced by *Dt*. These kernels could not have arisen by contamination, for stocks with the known *Dt* locus had never been obtained and thus no plants with this locus could have been present in the field. Furthermore, the stock having a_1 and *dt*, originally obtained from Rhoades, had been grown for several years. A number of sib crosses were made each year and no mutations of a_1 to A_1 were observed in the kernels on these ears.

The facts (1) that *Dt* is located in the heterochromatic knob of chromosome 9, (2) that the effect it produces can be recreated by subjecting chromosome 9 to the breakage-fusion-bridge cycle, (3) that *Ac* appeared in stocks that had undergone this cycle, and (4) that *Ac* and *Dt* are alike in their respective actions, all point to heterochromatin as the material composing *Ac*. The burst of new mutable loci which appeared in the self-pollinated progeny of plants that had been subjected to the chromosome type of breakage-fusion-bridge cycle becomes comprehensible if it is considered that the alterations in the quantity or structure of heterochromatic elements during this cycle were primarily responsible for the initial appearance of these mutable loci. This report has shown that, once such loci arise, other mutable loci arise through transposition of the inhibiting chromatin substances to other loci which in turn become mutable.

Why should altered heterochromatin be responsible for initiating such a chain of events? To answer this question, attention must be centered on the action of heterochromatin in the normal nucleus. That it is associated with the exchange of materials between nucleus and cytoplasm has been indicated.[9] Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action. There can be little question that transpositions of both *Ds* and *Ac* occur and that the time of their occurrence in the development of a tissue is under precise control. This control is determined by the number of *Ac* loci present and their organization and possibly their position in the chromosome complement. Is this transposition of heterochromatin? Is it a reflection of a process that normally occurs in nuclei? Is it responsible for controlling the rates and types of exchange that occur between nucleus and cytoplasm? Is it usually an orderly mechanism, which is related to the control of the processes of differentiation? If so, induced disturbances in quantity and organization of the heterochromatic elements of the chromosome could give rise to a series of alterations reflected both in chromosome structure and behavior and in genic reactions that could

markedly alter phenotypic expressions.[10] It is well known that the various knobs and centromeres may coalesce in the resting nuclei. This coalescence is also frequently observed both in the somatic and the meiotic prophases. Are the transpositions and the changes in state of *Ac* products of this coalescence? This is suspected because of the frequent transpositions of *Ac* from one chromosome to another.

It may be considered that these speculations with regard to heterochromatin behavior and function have been carried further than the evidence warrants. This may be true; but it cannot be denied that one basic kind of phenomenon appears to underlie the expression of variegation in maize. In many cases, there can be little question about the similarities in expression of variegation in Drosophila and maize. A heterochromatic element has repeatedly been found to be basically associated with the origin and expression of variegation in Drosophila. That a heterochromatic element likewise is responsible for the origin and behavior of variegation in maize has not been proved, although it is indicated, as the analysis of *Dt* has shown.

[1] Lewis, E. B., *Advances in Genetics*, **3**, 73–115 (1950).

[2] The symbols refer to genes affecting the parts of the plant as follows: *c*, aleurone pigment; *yg*, chlorophyll; *wx*, composition of starch in pollen and endosperm; a_2, aleurone pigment; *y*, starch composition of endosperm; *pyd*, chlorophyll.

[3] McClintock, B., PROC. NATL. ACAD. SCI., **28**, 458–463 (1942).

[4] The annual reports of the author, appearing in the Yearbooks of the Carnegie Institution of Washington, **41–48**, (1942–1949), contain more detailed summaries of some of the observations that are described in this paper.

[5] Catcheside, D. G., *J. Genet.*, **38**, 345–352 (1939); *Ibid.*, **48**, 31–42 (1947); *Ibid.*, **48**, 99–110 (1947).

[6] Rhoades, M. M., *Genetics*, **23**, 377–397 (1938). *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 138–155 (1941); PROC. NATL. ACAD. SCI., **31**, 91–95 (1945).

[7] Stern, C., *Genetics*, **21**, 625–730 (1936).

[8] McClintock, B., *Ibid.*, **26**, 234–282 (1941).

[9] Vanderlyn, L., *Bot. Rev.*, **15**, 507–582 (1949).

[10] This report deals only with the origin and behavior of mutable loci arising in these cultures. A number of other heritable changes are also arising. Many are associated with marked alterations in morphological characters.

GENERALIZED EISENSTEIN SERIES AND NON-ANALYTIC AUTOMORPHIC FUNCTIONS

By Richard Bellman

Department of Mathematics, Stanford University

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1. Introduction.—In a recent paper concerned with the uniqueness of Dirichlet series satisfying functional equations, Maass[4] introduced the concept of non-analytic automorphic functions. These are functions of two real variables, $f(x_1, x_2)$, possessing functional equations of the type

$$\begin{aligned} f(x_1, x_2 + c) &= f(x_1, x_2), \\ f(x_1', x_2') &= R(x_1, x_2) + s(x_1, x_2) f(x_1, x_2), \end{aligned} \tag{1}$$

where $x_1' + ix_2' = 1/(x_1 + ix_2)$.

These functions are connected with the zeta function of a quadratic number field, and their automorphic properties are deduced not as a direct consequence of the functional equations of the related Dirichlet series, nor as a consequence of the transformations of the associated theta functions, but, in a most interesting manner, as a result of the uniqueness of the solution of the partial differential equation satisfied by $f(x_1, x_2)$.

The purpose of this note is to indicate how a large class of non-analytic automorphic functions may be deduced from the fundamental analytic automorphic functions, the theta functions. Furthermore, the invariant character of these new functions will be demonstrated by deriving generalized Eisenstein series expansions for the functions. The associated Dirichlet series are the Epstein zeta functions.

Our basic tool is the class of functions, $W_a(x)$, defined by

$$W_a(x) = \int_0^\infty e^{-xv - 1/v} v^{-a} dv, \qquad Re(x) > 0, \tag{2}$$

together with its generalizations and analogs, which we discuss below.

The case $a = 3/2$ leads, with some non-essential changes, to the well-known formula,

$$e^{-\sqrt{x}} = \frac{1}{2\sqrt{\pi}} \int_0^\infty e^{-xv - 1/4v} v^{-3/2} dv, \tag{3}$$

while the case $a = 1$ introduces a cylinder function, used by Maass in the construction of his functions.

As a first simple application of the method, we have

$$\sum_{m,n=-\infty}^{\infty}{}' e^{-\pi\sqrt{x(m^2+n^2)}} = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^{\infty} \sum_{m,n=-\infty}^{\infty} e^{-vx^2(m^2+n^2)} e^{-1/4v} v^{-3/2} dv. \tag{4}$$

Applying the classical transformation formula, $\sum_{n=-\infty}^{\infty} e^{-n^2\pi t} = f(t) = t^{-1/2} f(1/t)$, this becomes

$$= \frac{1}{2\sqrt{\pi x^2}} \int_{-\infty}^{\infty} \sum_{m,n=-\infty}^{\infty} e^{-\pi(m^2+n^2)/x^2 v} e^{-1/v} v^{-3/2} dv, \qquad (5)$$

whence

$$\sum_{m,n=-\infty}^{\infty} e^{-\pi\sqrt{\pi(m^2+n^2)}} = 2x \sum_{m,n=-\infty}^{\infty} [4\pi(m^2+n^2)+x^2]^{-3/2}. \quad (6)$$

This application of (3) is due to Hardy,[3] where many relations pertaining to the functions $W_\alpha(x)$ are given. At the time this formula was first derived, as he himself noted subsequently, Hardy was not interested in any applications to number theory. When, afterwards,[2] he returned to (6) in connection with lattice points within a circle, he derived this formula in a different fashion. The possibilities of the method have not been fully explored, and we shall see that a slight extension of the method yields many interesting identities, identities which may be used to render obvious the automorphic behavior of the functions involved.

It will be clear from what follows that we may replace $m^2 + n^2$ by an arbitrary positive definite quadratic form in N variables. We will restrict ourselves to the simplest case in illustrating our method.

In place of the former series, we now consider

$$\sum_{m,n=-\infty}^{\infty} e^{-\pi x_1\sqrt{x(m^2+n^2)}+2imx_2+2inx_3} = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^{\infty} \sum_{m,n=-\infty}^{\infty} e^{-\pi x_1^2(m^2+n^2)v+2imx_2+2inx_3} e^{-1/v} v^{-3/2} dv. \quad (7)$$

Inserting the transformation formula for $\sum e^{-\pi m^2 v_1 + 2imv_2}$ and carrying out the indicated integration with respect to v as before, we now obtain, after making the slight change, $x \to x\sqrt{4\pi}$,

$$\sum_{m,n=-\infty}^{\infty} e^{-2x_1\sqrt{\pi^2(m^2+n^2)}+2imx_2+2inx_3} = x_1(4\pi)^{-3/2} \sum_{m,n=-\infty}^{\infty} [(m+x_2)^2+(n+x_3)^2+x_1^2]^{-3/2}. \quad (8)$$

If we now divide by x_1, take the partial derivatives of both sides of the equation with respect to x_1, divide again by x_1, transpose the term corresponding to $m = n = 0$ on the right-hand side to the left-hand side, set $x_1 = x_1'\sqrt{\mu^2+\nu^2}$, $x_2 = \mu x_2'$, $x_3 = \nu x_3'$, and then sum over all values of μ and ν apart from (0, 0), we obtain on the right-hand side a constant multiple of the generalized Eisenstein series,

$$\sum_{m,n,\mu,\nu}{}' [(m + \mu x_2)^2 + (n + \nu x_2)^2 + (\mu^2 + \nu^2)x_1^2]^{-7/4} \qquad (9)$$

($'$ indicates the $m = n = \mu = \nu = 0$ is excluded.) All these gyrations are due to the necessity of ensuring the convergence of the resulting expression, which does converge because the left-hand member converges.

Setting x_2 and x_3 equal, it is readily verified that we have a function satisfying (1) when $x_1 + ix_2 = 1/x_1' + ix_2'$.

2. In addition to the generalizations of the above result furnished by more general quadratic forms and the theta functions associated with algebraic number fields, an extensive generalization is obtained by replacing the class $W_s(x)$ of (2) above by the matrix functions

$$W_s(X) = \int e^{-\pi p(XV + V^{-1})} |V|^{-s} dV \qquad (1)$$

where the integration is over the Siegel space of nxn positive definite matrices V, X is a positive definite matrix, and $|V|$ represents the determinant of V. The functions obtained by the analog of the previous method are real analogs of the generalized Eisenstein series of Siegel.[5] All this will be discussed in detail in a subsequent work in which we hope also to determine the connection between these new functions and the problem of Hecke considered by Maass.

3. As noted above, to obtain non-analytic automorphic functions of the type of Maass, we use the function $W_1(x)$. This function is the case $n = 2$ of the general Voronoi function, $V_n(x)$, defined by

$$\int_0^\infty V_n(x)x^{s-1}dx = \Gamma(s)^n. \qquad (1)$$

This function was first introduced, I believe, by Voronoi in his classic memoir on the Dirichlet divisor problem. Using Mellin's inversion formula $V_n(x)$ may be represented by a complex integral. The analog of (2) of §1 also exists, namely,

$$V_n(x) = \int_0^\infty \cdots \int_0^\infty \frac{e^{-\pi x(t_1 \cdots t_n + 1/t_1 + 1/t_2 \cdots + 1/t_n}}{t_1 t_2 \cdots t_n} \pi dt_i. \qquad (2)$$

From (1) it follows readily that the function defined by

$$f_k(x) = \sum_{n=1}^{\infty} d_k(n) V_k(n^2x^2), \qquad Re(x^2) > 0, \qquad (3)$$

where $\zeta(s)^k = \sum_{n=1}^{\infty} d_k(n)n^{-s}$ possesses a functional equation connecting its value at x with its value at c_k/x, where c_k is a constant depending upon k. Furthermore, it may also be shown that

$$g_k(x) = \sum_{n=1}^{\infty} d_{2k}(n) V_k(n^2x^2) \qquad (4)$$

possesses an approximate functional equation, cf. reference 1, where this last result was used in connection with the mean value of $\zeta(s)$ on the critical line. The case $k = 1$ is due to Wigert.

It seems very difficult to establish corresponding results for

$$f_k(x, y) = \sum_{n=1}^{\infty} d_k(n) V_k(n^2x^2) e^{iny} \qquad (5)$$

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ARITHMETICAL PROPERTIES OF POLYNOMIALS ASSOCIATED WITH THE LEMNISCATE ELLIPTIC FUNCTIONS

By Morgan Ward

Department of Mathematics, California Institute, Pasadena

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1. I have studied elsewhere the arithmetical properties of certain polynomials associated with the real multiplication of elliptic functions.[1] Such polynomials include as a special case the function $U_n = (a^b - b)_n/(a - b)$ first systematically studied by Lucas[2] and Sylvester[3] when expressed as a polynomial in $P = a + b$ and $Q = ab$.

I have recently investigated the polynomials associated with the simplest type of complex multiplication of elliptic functions; namely, the so-called lemniscate case for which the period ratio τ has the value $i = \sqrt{-1}$ and the Weierstrass invariant g_2 is zero.

In the account which follows, the small greek letters α, ϵ, λ, μ, ν and π will be used for elements of the ring G of Gaussian integers. $\bar{\alpha}$ and $N\alpha$ denote the conjugate and norm of α in G. α is said to be odd, oddly even or totally even according as $N\alpha$ is congruent to one, two or zero modulo 4. The letter ϵ is reserved for denoting any one of the four units ± 1, $\pm i$ of the ring G.

2. Let u be a complex variable, and $\wp(u)$ the Weierstrass $\wp$-function formed with the invariants $g_2 = 4w$, $g_3 = 0$. Let $E_\mu = E_\mu(u)$ equal 1,

$\sqrt{\wp(u)}$ or $\wp'(u)$ according as μ is odd, oddly even or totally even. Finally let

$$\Psi_\mu = \Psi_\mu(u) = \sigma(\mu u)/\sigma(u)^{N\mu} \tag{1}$$

where $\sigma(u)$ is the Weierstrass sigma function. Then $\Psi_\mu + E_\mu$ is an even elliptic function with the same periods as $\wp(u)$. More specifically,

$$\Psi_\mu(u) = E_\mu(u)P_\mu(z, w) \tag{2}$$

where

$$P_\mu = P_\mu(z, w) = \sum_{r=0}^{q} \pi_r z^{q-2r} w^r \tag{3}$$

is a polynomial in $z = \wp(u)$ and $w = \frac{g_2}{4}$ whose coefficients π_r are Gaussian integers with $\pi_0 = \mu$. The degree q of P_μ in z depends in a simple way on $N\mu$. The arithmetical properties of these polynomials were the object of the investigation; (3) is the elliptic function analog of the cyclotomic polynomial $\frac{z^n - 1}{z - 1}$ associated with Lucas' U_n.

3. The arithmetical properties of the polynomials P_μ closely parallel the properties of Lucas' U_n. The main new feature of interest (not occurring in the real multiplication case) is a genuine double numerical periodicity when the free variables z and w are given fixed values in G, and the residues of the resulting sequence in G are considered for moduli in G. Indeed Lucas claimed in his fundamental paper and elsewhere to have discovered doubly periodic numerical functions connected with the elliptic functions, but he apparently published nothing on this subject.[4]

The Lucas polynomial U_n may be defined as the solution of a simple difference equation with prescribed initial values. The function Ψ_μ may be similarly defined as a solution of the difference equation

$$\Omega_{\mu+\nu}\Omega_{\mu-\nu} = \Omega_{\mu+1}\Omega_{\mu-1}\Omega_\nu^2 - \Omega_{\nu+1}\Omega_{\nu-1}\Omega_\mu^2 \tag{4}$$

with prescribed initial values; in particular, $\Psi_0 = 0$ and $\Psi_\epsilon = \epsilon$. (A table of the corresponding initial values of P_μ for small $N\mu$ is given at the close of the paper.)

Consequently, just as in the real multiplication case,[5] the polynomials P_μ may be defined purely algebraically as modified solutions of (4). On using this algebraic definition in conjunction with the function-theoretic definitions (1) and (2), the following results were obtained.

(i) *If z, w are indeterminates, the correspondence $\nu \to P_\mu(z, w)$ is a mapping of the ring G into the polynomial ring $G(z, w)$ which preserves*

division; that is ν divides μ in G implies that P_ν divides P_μ in $G(z, w)$. Furthermore,

$$P_\epsilon = \epsilon, \qquad P_{\epsilon\mu} = \epsilon P_\mu, \qquad P_{\bar{\mu}} = \bar{P}_\mu.$$

Therefore if μ is a rational integer, all the coefficients π_ν of P_μ are rational integers, and P_μ reduces to the polynomial of the real multiplication case studied in reference 1.

Let z_0, w_0 be fixed rational integers. Then $h_\nu = P_\nu(z_0, w_0)$ is a Gaussian integer and the correspondence $\nu \to h_\nu$ is a mapping of G into itself preserving division. Let π from now on denote a fixed Gaussian prime. An integer λ is called a zero of h_ν modulo π if $h_\lambda \equiv 0 (\mathrm{mod}\ \pi)$ and a rank of apparition of π in $\{h_\nu\}$ if $h_\lambda \equiv 0 (\mathrm{mod}\ \pi)$ but $h_\mu \not\equiv 0 (\mathrm{mod}\ \pi)$ for μ any proper divisor of λ.

(ii) *If π is odd, the zeros of the prime π in $\{h_\nu\}$ form an ideal m which is never the zero ideal.* Furthermore if λ is any rank of apparition of π in $\{h_\nu\}$, m is the principal ideal determined by λ.

(iii) *If π is an odd complex Gaussian prime, then*[6]

$$P_\mu(z, w) \equiv P_\mu(0, w) \pmod{\pi}.$$

(iv) *The sequence $\{h_\nu\}$ becomes numerically periodic modulo π. The moduli of its periods is contained in the ideal m of its zeros modulo π.*

(v) *Given a specific term h_λ of $\{h_\nu\}$, the only odd primes π which can have rank of apparition λ in $\{h_\nu\}$ are either divisors of λ, or primes for which the polynomial $P_\lambda(z, w)$ splits completely into linear factors or completely into quadratic factors in the residue class ring $G(z, w)/(\pi)$.* Such primes lie in arithmetical progressions whose common constant difference is a function of λ alone.[7]

(v) generalizes the well-known result of Lucas and Sylvester that if P and Q are rational integers, all primitive prime divisors of U_l are either divisors of l or of the form $kl \pm 1$.

The first few polynomials P_μ are as follows: $P_0 = 0$, $P_1 = 1$, $P_i = i$, $P_{1+i} = 1 + i$, $P_2 = 2$, $P_3 = 3z^4 - 6wz^2 - w^2$, $P_{1+2i} = (1 + 2i)z^3 - w$, $P_{3+i} = (3 + i)z^4 - 2(1 + 3i)wz^2 + (3 + i)w^2$. All the remaining P_μ can be calculated from the recursion (4) and the relations $P_\mu = \bar{P}_\mu$, $P_{\epsilon\mu} = \epsilon P_\mu$.

Qualitatively similar results hold for the polynomials associated with any complex multiplication of $\mathcal{P}(u)$.[8]

A more complete account of these and other results with proofs will be published elsewhere.

[1] *Am. J. Math.*, **70**, 31–74 (1948). Various algebraic properties of these polynomials are developed in Halphen's treatise on elliptic functions.

[2] *Ibid.*, **1**, 184–240, 289–321 (1878).

[3] *Ibid.*, **2**, 357–380 (1879).

[6] In particular, Lucas stated to C. A. Laisant that there was a remarkable connection between his doubly periodic numerical functions and Fermat's last theorem. See Bell, E. T., *Bull. Am. Math. Soc.*, **29**, 401–406 (1923). The crux of the matter is to understand what Lucas meant by "double periodicity." Since the modules of the ring of integers are all principal ideals, no numerical function of the rational integer n can be doubly periodic. The simplest case in which double periodicity in the usually understood sense can occur is for numerical functions over the ring of Gaussian integers.

[6] See Chapter V of reference 1.

[6] Due to Eisenstein for the Jacobian lemniscate polynomials and used by him to prove the biquadratic reciprocity law. See his *Math. Abh.*, third paper or *J. Math. (Crelle)*, **30**, 184–187 (1846).

[7] This result follows from Abel's theorem that the Galois group of the equation $P_q(z, w) = 0$ in z is commutative and of order q.

[8] The equi-harmonic case when the period ratio τ is a complex cube root of unity and the invariant g_3 vanishes is being studied in detail by Lincoln K. Durst.

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